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Properties of Crystalline L-Ornithine:α-Ketoglutarate δ-Aminotransferase from *Bacillus sphaericus*

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The distribution of bacterial L-ornithine:α-ketoglutarate δ-aminotransferase (L-ornithine:2-oxo-acid aminotransferase [EC 2.6.1.13]) was investigated, and *Bacillus sphaericus* (IFO 3525) was found to have the highest activity of the enzyme, which was inducibly formed by addition of L-ornithine or L-arginine to the medium. L-Ornithine:α-ketoglutarate δ-aminotransferase, purified to homogeneity and crystallized from *B. sphaericus*, had a molecular weight of about 80,000 and consisted of two subunits identical in molecular weight (41,000) and in amino-terminal residue (threonine). The enzyme exhibited absorption maxima at 278, 343, and 425 nm and contained 1 mol of pyridoxal 5'-phosphate per mol of enzyme. The formyl group of pyridoxal 5'-phosphate was bound through an aldimine linkage to the ε-amino group of a lysine residue of the protein. The enzyme-bound pyridoxal 5'-phosphate, absorbing at 425 nm, was released by incubation with phenylhydrazine to yield the catalytically inactive form. The inactive enzyme, which was reactivated by addition of pyridoxal 5'-phosphate, still had a 343-nm peak and contained 1 mol of a vitamin B_6 compound. The holoenzyme showed positive circular dichroic bands at 340 and 425 nm, whereas the inactive form had no band at 425 nm. The enzyme was highly specific for L-ornithine and α-ketoglutarate and catalyzed δ-transamination between them to produce L-glutamate and L-glutamate γ-semialdehyde, which was spontaneously converted to Δ^1-pyrroline-5-carboxylate. The enzyme activity was significantly affected by nonsubstrate amino acids, amines, and carbonyl reagents.

We have studied bacterial ω-aminotransferases, such as L-lysine:α-ketoglutarate ε-aminotransferase (27), taurine:α-ketoglutarate aminotransferase (32), and ω-amino acid:pyruvate aminotransferase (40). L-Ornithine:α-ketoglutarate δ-aminotransferase (ornithine δ-aminotransferase, L-ornithine:2-oxo-acid aminotransferase [EC 2.6.1.13]), another ω-aminotransferase, was discovered first in mammalian tissues (21). The enzyme has been found widely in animals (10, 21, 25, 31), higher plants (30), and microorganisms (5, 18, 26, 33). Rat liver ornithine δ-aminotransferase has been studied enzymologically in detail (10, 20, 24), but only little attention has been given to the bacterial enzyme.

Recently, we have purified to homogeneity and crystallized ornithine δ-aminotransferase from *Bacillus sphaericus* (38). In the present paper, enzymological and physicochemical properties of the crystalline enzyme are described.

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MATERIALS AND METHODS

Materials. Pyridoxal 5'-phosphate (pyridoxal-P) and L-amino acids were obtained from Kyowa Hakko Kogyo and Ajinomoto, respectively, Tokyo, Japan; α-keto acids, pyridoxal, pyridoxamine, pyridoxamine 5'-phosphate, pyridoxine 5'-phosphate, and sodium dodecyl sulfate prepared especially for protein research were from Nakarai Chemicals, Kyoto, Japan; standard proteins for molecular weight determination and 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) chloride were from Sigma Chemical Co., St. Louis, Mo.; Sephadex G-25 and Sephadex G-150 were from Pharmacia, Uppsala, Sweden; and 3-methyl-2-benzothiazolone hydrazone hydrochloride was from Aldrich Chemical Co., Inc., Milwaukee, Wis. Δ^1-Pyrroline-2-carboxylate and Δ^1-pyrroline-5-carboxylate were synthesized from D-proline with D-amino acid oxidase and from L-ornithine with L-lysine:α-ketoglutarate ε-aminotransferase, respectively (27). N^{ϵ}-Pyridoxyllysine was kindly provided by Y. Morino, Kumamoto University, Kumamoto, Japan. The other chemicals were analytical-grade reagents.

Microorganisms and conditions of culture. *B. sphaericus* IFO 3525 and other strains were grown in a medium containing 0.4% L-arginine hydrochloride, 0.5% peptone, 0.2% glycerol, 0.2% KH_2PO_4, 0.2%

K_2HPO_4, 0.2% NaCl, and 0.05% yeast extract. The pH of the medium was adjusted to 7.2 with 2 N NaOH. The bacteria were grown aerobically in 500-ml flasks containing 150 ml of the medium on a reciprocating shaker or in a 200-liter Marubishi fermentor jar at 28°C for 18 to 20 h. The cells were harvested by centrifugation and were washed twice with 0.85% NaCl and subsequently with 10 mM potassium phosphate buffer (pH 7.2) containing 0.01% 2-mercaptoethanol. The washed cells were stored at −20°C until use.

Enzyme preparation. Ornithine δ-aminotransferase was purified from a cell-free extract of *B. sphaericus* IFO 3525 and crystallized as described previously (38). The specific activity of the crystalline preparation was approximately 53 U/mg of protein.

Enzyme and protein assays. The enzyme was assayed by determination of Δ^1-pyrroline-5-carboxylate or L-glutamate formed, as reported previously (38). One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmol of Δ^1-pyrroline-5-carboxylate or L-glutamate per min. Specific activity is expressed as units per milligram of protein. Protein was determined by the method of Lowry et al. (13) with crystalline bovine serum albumin as a standard. Protein concentration of the purified enzyme was derived from absorbance at 278 nm. The absorbance coefficient ($A^{1\%}_{1cm}$ = 11.2), obtained by absorbance and dry weight determinations, was used throughout.

Amino terminal analysis. NH_2-terminal amino acids were determined by the dansyl chloride method (7, 36). A 0.35% protein solution (0.2 ml, dialyzed with 0.1 M sodium phosphate buffer [pH 7.2] containing 4 M urea) was incubated with 0.1 ml of dansyl chloride solution (0.5%, wt/vol, in acetone) at 37°C for 30 min. After hydrolysis of the dansylated protein in 6 N HCl at 110°C for 14 h, the dansyl amino acid in the hydrolysate was identified by two-dimensional chromatography (12) on polyamide thin-layer sheets.

Amino acid composition. Amino acid analyses were performed according to the method of Spackman et al. (29) with a Hitachi model 835 amino acid analyzer. The enzyme was dialyzed against 1 mM potassium phosphate buffer (pH 7.2) for 24 h. Four samples of 250 μg of the enzyme were lyophilized in acid-washed tubes. One sample was treated with performic acid. Hydrolysis was performed in 1 ml of 6 N HCl under reduced pressure for 24, 48, or 72 h at 110°C. The hydrolysates were evaporated to dryness under reduced pressue and subjected to amino acid analysis in duplicate. Cysteine and cystine were determined as cysteic acid after oxidation of protein with performic acid and hydrolysis (19). Tryptophan and tyrosine were determined spectrophotometrically by the method of Edelhoch (4).

Spectrophotometry. Absorption and circular dichroism spectra were taken with a Union Giken SM-401 recording spectrophotometer and a Union Giken Mark III-J dichrograph equipped with a Sord M-223 computer system, respectively.

RESULTS

Bacterial distribution of ornithine δ-aminotransferase. Screening was carried out to find bacterial strains that would produce a high activity of ornithine δ-aminotransferase. Enzyme activity was determined by measurement of the amount of Δ^1-pyrroline-5-carboxylate formed with the cell-free extracts. The enzyme was found to occur in various strains of bacteria (Table 1). *B. sphaericus* IFO 3525, in which ornithine δ-aminotransferase occurred most abundantly, was chosen for the purpose of purification of the enzyme, though high activity was found also in *Erwinia aroideae*, *Staphylococcus aureus*, and strain IFO 3306.

Induction of enzyme by L-ornithine and L-arginine. Extracts of *B. sphaericus* prepared from cells grown in the absence of added L-ornithine or L-arginine showed only low activity of the enzyme. Figure 1 shows the effects of L-ornithine or L-arginine concentrations in the growth medium on the formation of ornithine δ-aminotransferase. Addition of L-ornithine at an initial concentration of 0.3% caused a 6.6-fold

TABLE 1. *Distribution of ornithine δ-aminotransferase activity in various bacterial strains*[a]

Strain	Ornithine δ-aminotransferase sp act (U/mg of protein)
Escherichia coli Crookes ICR 0010	0.10
Escherichia coli E-9 ICR 0040	0.02
Escherichia coli Najjar IAM 1239	0.35
Klebsiella pneumoniae IFO 3319	0.06
Enterobacter cloacae IFO 3320	0.04
Erwinia carotovora subsp. *carotovora* IFO 3830	0.37
Proteus vulgaris IFO 3045	0.02
Proteus mirabilis IFO 3849	0.02
Alcaligenes faecalis ICR 0800	0.08
Achromobacter sp. strain ICR 0890	0.14
Bacillus cereus IFO 3001	0.16
Bacillus sp. IFO 3028	0.21
Bacillus subtilis IFO 3009	0.29
Bacillus subtilis IAM 1069	0.33
Bacillus sphaericus IFO 3525	0.67
Bacillus natto Sawamura ICR 1350	0.31
Agrobacterium tumefaciens ICR 1600	0.16
Agrobacterium radiobacter ICR 1610	0.20
Micrococcus sp. strain IFO 3242	0.25
Staphylococcus aureus IFO 3060	0.55
Micrococcus luteus IFO 3064	0.19
Strain IFO 3306[b]	0.60
Corynebacterium pseudodiphtheriticum ICR 2210	0.02
Pseudomonas reptilivora IFO 3461	0.10
Chromobacterium iodinum IFO 3558	0.16
Brevibacterium ammoniagenes IFO 1202	0.12
Brevibacterium ammoniagenes IAM 1641	0.10

[a] Conditions of culture and enzyme assay are given in the text.

[b] *Corynebacterium*-like, but not fully identified.

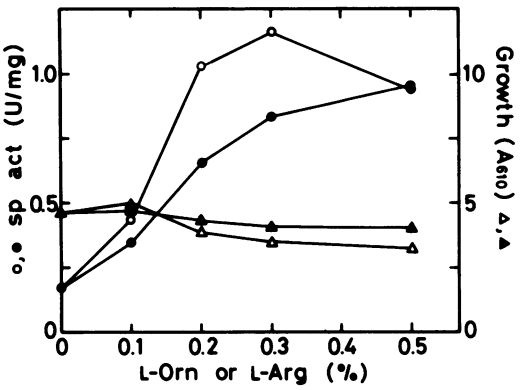

FIG. 1. *Effect of* L-*ornithine (*L-*Orn)* (○, △) *or* L-*arginine (*L-*Arg)* (●, ▲) *added to the culture medium on ornithine δ-aminotransferase activity. B. sphaericus IFO 3525 was grown in the medium supplemented with the indicated concentrations of* L-*Orn or* L-*Arg. Bacterial growth was determined by measuring the turbidity of culture suspensions at 610 nm (A_{610}). Enzyme activity in cell-free extracts was determined as described in the text.*

increase in the activity, and addition of L-arginine at an initial concentration of 0.5% caused a 6.2-fold increase. This showed that the bacterial ornithine δ-aminotransferase was produced inducibly. A higher concentration of L-arginine than L-ornithine was required for maximal production of the enzyme activity. We found that arginase was also induced in the cells by the addition of L-arginine. The specific activity of arginase in cell-free extracts of *B. sphaericus* grown in the medium containing 0.4% L-arginine was 3.0 μmol/min per mg of protein (M. Yasuda, K. Tanizawa, S. Toyama, and K. Soda, unpublished data). Thus, when the cells were grown in the L-arginine-containing medium, ornithine δ-aminotransferase probably was induced by L-ornithine produced from L-arginine by the cellular arginase.

Purity and molecular weight. The crystalline enzyme of *B. sphaericus* was shown to be homogeneous upon ultracentrifugation and disc gel electrophoresis, as reported in a previous paper (38). The sedimentation coefficient ($S^0_{20,w}$) of the enzyme was 5.3 S. The molecular weight of the enzyme was determined to be about 85,000 by the sedimentation equilibrium method (38). A molecular weight of about 80,000 was also obtained by the Sephadex G-150 gel filtration method (1), with alcohol dehydrogenase (M_r 126,000), bovine serum albumin (M_r 68,000), chymotrypsinogen A (M_r 25,000), and cytochrome *c* (M_r 12,400) as standard proteins.

Structure of subunit. The subunit structure of the enzyme was examined by disc gel electrophoresis. The enzyme was incubated with 1.0% sodium dodecyl sulfate in 50 mM sodium phosphate buffer (pH 7.2) containing 1.0% 2-mercaptoethanol and 25% glycerol at 37°C for 12 h. The treated enzyme preparation was subjected to electrophoresis in the presence of 0.1% sodium dodecyl sulfate (35). There was a single band of stained protein. To determine the molecular weight of the polypeptide in this band, we ran a series of marker proteins treated in the same manner: bovine serum albumin (M_r 68,000), glutamate dehydrogenase (M_r 50,000), egg albumin (M_r 43,000), chymotrypsinogen A (M_r 25,000), and cytochrome *c* (M_r 12,400). The molecular weight was calculated to be approximately 41,000 from a semilogarithmic plot of molecular weight against mobility, indicating that the enzyme consisted of two subunits identical in molecular weight. The subunit identity was examined by NH_2-terminal amino acid analysis. Only threonine was detected as the NH_2-terminal amino acid of the enzyme by the dansyl chloride method, suggesting that the enzyme was composed of two identical subunits.

Amino acid composition. Amino acid analyses of the purified ornithine δ-aminotransferase (Table 2) revealed the presence of all the common amino acids, among which glutamic acid, leucine, alanine, glycine, and aspartic acid were the most plentiful. Although the number of amide nitrogens was not determined, the relatively high content of glutamic and aspartic acids suggested that the enzyme was an acidic protein. The amino acid composition of rat liver enzyme (16, 24), normalized to its subunit molecular weight, 45,000 (24), is included in Table 2 for comparison. A few distinct differences between the two enzymes were found in the residue numbers of proline, tryptophan, and glutamic acid. However, a comparison of the two enzymes on a mole percent basis revealed their considerable similarity; a statistical analysis of the amino acid compositions by the method of Harris et al. (8) yielded a low deviation function ($D = 0.0734$).

Absorption spectrum of enzyme and reduction with sodium borohydride. The holoenzyme exhibited absorption maxima at 278, 343, and 425 nm at pH 7.4, with molar absorption coefficients of 92,500, 11,400, and 6,000, respectively (Fig. 2A, curve a). The addition of L-ornithine to the enzyme solution at pH 7.4 caused a decrease in absorbance at 425 nm and an increase at about 340 nm. The occurrence of the peak at 425 nm suggested that the formyl group of pyridoxal-P formed an aldimine linkage with an amino group of the protein by analogy with the bound coenzyme of other pyridoxal-P enzymes thus far studied.

Reduction of the enzyme with sodium boro-

TABLE 2. *Amino acid composition of ornithine δ-aminotransferase*

Amino acid	*B. sphaericus* enzyme					Rat liver enzyme[a]	
	No. of residues (mol)[b]				mol%	No of residues (mol)[c]	mol%
	24 h	48 h	72 h	Avg. or extrapolated values[d]			
Aspartic acid	29.3	26.6	30.7	28.9 (29)	7.78	25.4	7.61
Threonine	16.0	15.2	13.4	17.1[e] (17)	4.60	20.0	5.99
Serine	12.4	11.1	9.2	13.9[e] (14)	3.74	16.0	4.79
Glutamic acid	51.6	50.5	51.1	51.1 (51)	13.75	33.5	10.03
Proline	8.4	8.0	8.3	8.2 (8)	2.21	22.6	6.77
Glycine	35.3	31.4	30.8	32.3 (32)	8.69	29.4	8.81
Alanine	36.8	37.9	39.3	38.0 (38)	10.22	27.8	8.33
Half-cystine	5.0			5.0[f] (5)	1.35	5.0	1.50
Valine	15.3	18.0	19.0	19.0[g] (19)	5.11	23.4	7.01
Methionine	7.3	7.4	7.6	7.4[h] (7)	1.99	7.4	2.22
Isoleucine	22.0	24.7	25.3	25.3[g] (25)	6.81	21.0	6.29
Leucine	38.4	37.6	38.8	38.8[g] (39)	10.44	32.9	9.85
Tyrosine	9.5	10.4	10.3	10.1[i] (10)	2.72	13.0	3.89
Phenylalanine	15.4	14.2	15.0	14.9 (15)	4.01	10.4	3.11
Lysine	25.1	26.5	26.3	26.0 (26)	6.99	18.9	5.66
Histidine	5.3	5.6	5.3	5.4 (5)	1.45	5.8	1.74
Arginine	17.6	18.4	17.9	17.9[g] (18)	4.82	17.2	5.15
Tryptophan				12.4[j] (12)	3.34	4.2	1.26

[a] Data adapted from reference 24.
[b] Residues per subunit (M_r = 41,000).
[c] Residues per subunit (M_r = 45,000).
[d] Numbers in parentheses represent numbers of proposed amino acid residues per subunit.
[e] Obtained by extrapolation to zero hydrolysis time.
[f] Determined as cysteic acid after performic acid oxidation.
[g] Value given by the 72-h hydrolysis time.
[h] Also determined to be 6.9 mol/mol of subunit as methionine sulfone after performic acid oxidation.
[i] Also determined spectrophotometrically to be 10.2 mol/mol of subunit.
[j] Determined spectrophotometrically.

hydride by the dialysis method of Matsuo and Greenberg (14) affected both the absorption spectrum (Fig. 2A, curve b) and the activity. The reduced enzyme was catalytically inactive, and the addition of pyridoxal-P did not reverse the inactivation. These results show that the borohydride reduces the aldimine linkage to yield the aldamine bond. To identify the amino acid residue to which pyridoxal-P binds in the enzyme, the hydrolysate of the $NaBH_4$-reduced enzyme was examined by paper electrophoresis according to the method of Yonaha et al. (39). The fluorescent amino acid derivative in the hydrolysate was identified with the authentic N^{ϵ}-pyridoxyllysine.

Resolution and reconstitution of enzyme. Pyridoxal-P was required for maximum activity of the enzyme. Approximately 18% of the pyridoxal-P was removed from the enzyme by dialysis against 10 mM potassium phosphate buffer (pH 7.4) for 18 h. Full resolution of the enzyme was performed as follows. The enzyme was incubated with 50 mM phenylhydrazine (pH 7.4) at 37°C for 2 h, followed by gel filtration on a Sephadex G-25 column (0.9 by 40 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.4). The enzyme thus treated had no activity in the absence of added pyridoxal-P and no longer exhibited an absorption maximum at 425 nm, but had a maximum at 343 nm (Fig. 2A, curve c). Activity was fully restored by addition of either 10 μM pyridoxal-P or 50 μM pyridoxamine 5′-phosphate. Pyridoxal, pyridoxamine, and pyridoxine 5′-phosphate neither restored the activity nor acted as inhibitors. The Michaelis constants were estimated as 0.26 μM for pyridoxal-P and 3.6 μM for pyridoxamine 5′-phosphate. The active enzyme reconstituted with pyridoxal-P showed the same spectrum as the native holoenzyme.

Pyridoxal-P content. The enzyme solution (2.4 mg in 0.5 ml) was desalted through a Sephadex G-25 column with deionized water and then treated with 1.0 ml of 0.1 N HCl at 37°C for 30 min to release the bound pyridoxal-P. The amount of pyridoxal-P was measured by the phenylhydrazine method (34) and the 3-methyl-2-benzothiazolone hydrazone hydrochloride method (28). An average value of 1 mol of pyridoxal-P per 85,000 g of protein was obtained.

This indicated that 1 mol of pyridoxal-P was bound to 1 mol of the enzyme protein in the holoenzyme. The inactive form of the enzyme obtained by treatment with phenylhydrazine was examined by both methods in the same way. No appreciable amount of pyridoxal-P was found in the inactive enzyme. To determine the bound chromophore absorbing at 343 nm, the phenylhydrazine-treated enzyme (1.7 mg) was hydrolyzed in 0.055 N HCl at 110°C for 6 h and analyzed by the fluorometric method of Bonavita (2) and also by the microbiological method with *Saccharomyces carlsbergensis* (9). About 1 mol of vitamin B_6 compound was determined by the microbioassay per mol of the inactive enzyme, whereas the result of the fluorometric assay with KCN indicated the absence of pyridoxal. Nearly 2 mol of vitamin B_6 compound was detected per mol of the active holoenzyme when examined also by the microbioassay. Thus, the bacterial ornithine δ-aminotransferase contained 1 mol of pyridoxal-P, with λ_{max} at 425 nm, and 1 mol of another vitamin B_6 derivative, with λ_{max} at 343 nm.

The 343-nm peak of the enzyme was not shifted by addition of an amino acceptor, α-ketoglutarate, suggesting that the absorption was not derived from pyridoxamine 5'-phosphate. The 343-nm chromophore was bound tightly to the enzyme protein; the enzyme still showed an absorption peak at about 340 nm after it was incubated with 1% sodium dodecyl sulfate, 6 M guanidine hydrochloride, 8 M urea, or 1 N HCl.

Circular dichroism spectra. Circular dichroic spectra of the holoenzyme and the apoenzyme obtained by the phenylhydrazine treatment as described above were measured at pH 7.4 (Fig. 2B). The native holoenzyme exhibited a negative circular dichroic extremum at 220 nm and positive ones at 280, 343, and 425 nm, which correspond to the absorption maxima of the enzyme. The circular dichroism band at 343 nm was greater than the band at 425 nm. The apoen-

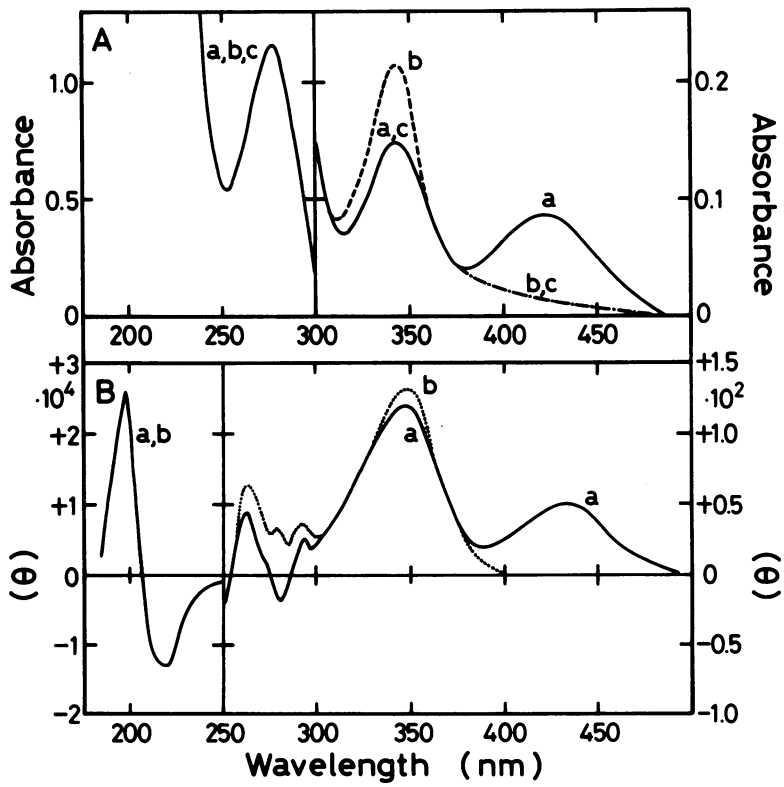

FIG. 2. *Absorption (A) and circular dichroic (B) spectra of ornithine δ-aminotransferase. (A) Curve a, Holoenzyme in 20 mM potassium phosphate buffer (pH 7.4); curve b, holoenzyme reduced with $NaBH_4$ and dialyzed against the same buffer; curve c, apoenzyme prepared as described in the text in 50 mM potassium phosphate buffer (pH 7.4). (B) Circular dichroic spectra of holoenzyme (curve a) and apoenzyme (curve b) measured in 20 mM potassium phosphate buffer (pH 7.4) in a cuvette at 25°C.*

zyme spectrum was similar to the holoenzyme spectrum except for the optical inactivity in the 400- to 480-nm region. α-Helix content of the enzyme was calculated to be approximately 39% by the method of Greenfield and Fasman (6).

Substrate specificity. The enzyme catalyzed almost exclusively the transamination between L-ornithine and α-ketoglutarate, as reported previously (38). Other amino acids and amines tested were not substrates, although β-lysine and L-lysine were slightly transaminated by the enzyme with α-ketoglutarate, with relative activities of 100 for L-ornithine, 1.9 for β-lysine, and 0.3 for L-lysine. The product from L-ornithine reacted with *o*-aminobenzaldehyde and ninhydrin to develop orange and yellow colors, respectively, in the same manner as reported previously (27). This suggested that a heterocyclic form of the keto analog of ornithine, i.e., Δ^1-pyrroline-2-carboxylate or Δ^1-pyrroline-5-carboxylate, was produced. To identify the reaction products, a reaction mixture containing L-ornithine, α-ketoglutarate, and 20 μg of enzyme was incubated at 37°C for 30 min. After deproteinization by addition of 0.2 ml of 50% trichloroacetate followed by centrifugation, the supernatant solution was adjusted to about pH 2.0 and analyzed with an automatic amino acid analyzer. The reaction products were identified as glutamic acid and Δ^1-pyrroline-5-carboxylate (Fig. 3). This result indicated the occurrence of δ-transamination between L-ornithine and α-ketoglutarate to produce glutamate-γ-semialdehyde, which spontaneously cyclized to form Δ^1-pyrroline-5-carboxylate. When glutamate produced was incubated with glutamate dehydrogenase from bovine liver (22), it was quantitatively oxidized to α-ketoglutarate; the L-isomer of glutamate was formed from α-ketoglutarate.

Amino acceptor specificity also was investigated with a reaction system containing L-ornithine as an amino donor and an excess amount of enzyme (150 μg). In addition to α-ketoglutarate (relative activity, 100), pyruvate (0.6), glyoxylate (0.5), and oxaloacetate (0.5) served as amino acceptors, though very slightly.

Inhibitors. Various compounds were investigated for their inhibitory effects on enzyme activity. The enzyme was inhibited 80% after a 20-min incubation with β-lysine, a poor substrate (as described above), at a concentration of 10 mM. Some of the other nonsubstrate amino acids and amines tested also were inhibitory at concentrations of 10 mM (e.g., L-lysine, 35% inhibition; L-arginine, 28%; L-valine, 35%; cadaverine, 57%; and putrescine, 63%). Hydroxylamine (94% inhibition at 1 mM), carboxymethoxylamine (96%), L-cycloserine (90%), 3-methyl-2-benzothiazolone hydrazone hydrochloride (78%),

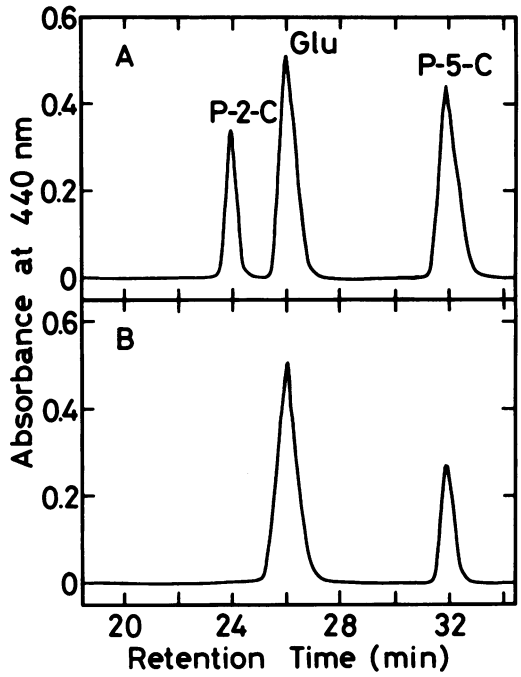

FIG. 3. *Ion-exchange chromatography of the reaction product formed from L-ornithine and α-ketoglutarate. A reaction mixture containing 20 μmol of L-ornithine, 20 μmol of α-ketoglutarate, 0.5 μmol of pyridoxal-P, 60 μmol of N-tris(hydroxymethyl)-methylglycine buffer (pH 8.5), and 20 μg of enzyme in a final volume of 1.0 ml was incubated at 37°C for 30 min. The reaction products were analyzed with a JEOL JLC-6AH amino acid analyzer (a JEOL, LCR-2 column [0.5 by 45 cm] at 50°C in 0.45 N citrate buffer, pH 4.25). (A) Authentic Δ^1-pyrroline-2-carboxylate (P-2-C), glutamate (Glu), and Δ^1-pyrroline-5-carboxylate (P-5-C); (B) reaction products.*

and phenylhydrazine (60%), which are typical inhibitors for pyridoxal-P enzymes, strongly inhibited the enzyme. The effect of each inhibitor was fully reversed by addition of pyridoxal-P (1.6 mM). Thiol reagents, e.g., *p*-chloromercuribenzoate and *N*-ethylmaleimide, and thiol compounds, e.g., 2-mercaptoethanol and dithiothreitol, did not show appreciable influences on the activity.

DISCUSSION

L-Ornithine plays a pivotal role in arginine and proline metabolism in mammals and microorganisms, as well as in higher plants. The conversion of L-ornithine to Δ^1-pyrroline-5-carboxylate is catalyzed by ornithine δ-aminotransferase, as demonstrated with enzyme systems of mammalian liver (31), *Neurospora crassa* (33), and *Saccharomyces cerevisiae* (18). In contrast, a recent study (17) has shown that the α-amino

group of L-ornithine is released in the biosynthesis of L-proline from L-ornithine in higher plants; Δ^1-pyrroline-2-carboxylate is produced from L-ornithine by ornithine α-aminotransferase. We have demonstrated in the present work that ornithine δ-aminotransferase from *B. sphaericus* catalyzes the δ-transamination between L-ornithine and α-ketoglutarate to produce L-glutamate and glutamate-γ-semialdehyde, which is intramolecularly dehydrated and cyclized to yield Δ^1-pyrroline-5-carboxylate. The enzyme was inducibly formed by addition of L-ornithine or L-arginine to the medium. Therefore, the bacterial enzyme probably functions in the degradation of L-arginine. The initial enzyme of this pathway, arginase, also was induced by L-arginine. This suggests that the bacterial arginine degradation involves the same enzyme system and metabolic control as that of a yeast, *S. cerevisiae* (3, 37).

On the basis of the characteristic spectrum of the purified enzyme, and also the isolation of N^{ϵ}-pyridoxyllysine from the hydrolysate of borohydride-reduced enzyme, it is likely that pyridoxal-P is bound to an ε-amino group of a lysine residue of the protein through an aldimine linkage. The determination of cofactor content of the enzyme shows that 1 mol of pyridoxal-P and 1 mol of another unknown form of vitamin B_6 compound are bound per mol of enzyme (85,000 g). The bound pyridoxal-P, with an absorption maximum at 425 nm, participates in the catalysis, and it is converted into pyridoxamine 5′-phosphate by incubation with the amino donor, L-ornithine. The chemical structure, the mode of binding to the enzyme protein, and the role in the catalytic action of the vitamin B_6 derivative absorbing at 343 nm remain unsettled, though it was shown that the substance is neither pyridoxal-P nor pyridoxamine 5′-phosphate. Sanada et al. (23, 24) reported that rat liver ornithine δ-aminotransferase also contains an unknown B_6 derivative with an absorption maximum at about 330 nm and suggested that the B_6 functions in maintaining the quaternary structure of the enzyme.

The bacterial ornithine δ-aminotransferase activity is affected by several kinds of amino acids and amines. It has been reported that the mammalian enzyme also is inhibited by branched-chain amino acids, such as L-valine and L-isoleucine (15, 31), and is strongly regulated in vivo by feedback inhibition (11). The inhibition of the bacterial enzyme by nonsubstrate amino acids and amines may, therefore, imply the existence of regulation of the enzyme in bacteria.

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